

The Genus *Hymenasplenium* (Aspleniaceae) in Cuba, Including New Combinations for the Neotropical Species

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ABSTRACT.—Morphological, cytological and taxonomical analyses of *Asplenium delitescens* and *A. laetum* in Cuba resulted in the recognition of both species belonging to the genus *Hymenasplenium* Hayata. The combinations of the Neotropical species to this genus are presented. Some remarks on gametophyte morphology of *H. delitescens* are included.

KEY WORDS.—*Hymenasplenium*, Cuba, new combinations

Hymenasplenium was briefly described in Japanese by Hayata (1927), citing *Asplenium unilaterale* Lam. (from Tropical Africa, Southeastern Asia, Japan, Polynesia north to Hawaii, Iwatsuki, 1975), as the type species, based on the differences in the stelar morphology of rhizome. The stelar morphology of *H. unilaterale* (Lam.) Hayata and its allied species was studied by Iwatsuki and Kato (1975). They proposed that the dorsiventral stele anatomy of this group might have evolved from the radial dictyostele typical of *Asplenium* by elongation of the rhizome, probably as an adaptation to the petrophytic habitat. However, they considered it inappropriate to separate *Hymenasplenium* based exclusively on this feature. For that reason, Iwatsuki (1975) proposed to treat *Hymenasplenium* as a section within *Asplenium*.

Murakami (1992) studied the stelar structure of Neotropical species of sect. *Hymenasplenium* and concluded the basic stelar structure was identical to the Old World Tropic species. Later, in the monograph for this group in the Neotropics, Murakami and Moran (1993) followed the criteria of Iwatsuki (1975) of treating *Hymenasplenium* as a section of *Asplenium*.

The first molecular analysis conducted for the Neotropical species of sect. *Hymenasplenium* revealed that this group is a distinct and coherent clade that is sister to the rest of *Asplenium* (Murakami and Schaal, 1994). Subsequently, Murakami (1995) published a review of the studies on *Hymenasplenium*. In the presented molecular phylogenetic tree all paleo- and neotropical species formed a monophyletic group, which lead Murakami (1995) to propose again the recognition of *Hymenasplenium* as a genus, although without doing the formal combination of the species names. These studies did not include West Indian samples. Succeeding works on molecular data confirmed the

monophyletic status of *Hymenasplenium* (Murakami *et al.*, 1999; Gastony and Johnson, 2001; Schneider *et al.*, 2004). Schneider *et al.* (2004) also pointed out that no hybrids have been found between *Hymenasplenium* and *Asplenium* species, although hybrids are otherwise common in Aspleniaceae.

The genus *Hymenasplenium* is defined by the unusual dorsiventral vascular system in long creeping rhizomes, swollen petiole bases and a chromosome number of $x=39$, 38, which differ from the radially symmetrical steles, non swollen petiole bases and chromosome number of $x=36$ typical of *Asplenium* (Murakami and Moran, 1993).

In the taxonomic treatment of Aspleniaceae for the "Flora de la República de Cuba", we recognized only *Asplenium* L. and *Schaffneria* Fée ex T. Moore (Sánchez and Regalado, 2003). We recorded two Cuban species of sect. *Hymenasplenium*, *Asplenium delitescens* (Maxon) L. D. Gómez and *A. laetum* Sw. We decided to treat both species within *Asplenium* because we could not reliably verify the chromosome numbers of both species. The only cytological data so far available for *Asplenium laetum* was a report of a sexual diploid cytotype from Jamaica with $2n=72$ (Walker, 1966), which corresponds with chromosome numbers found in *Asplenium* (Murakami and Moran, 1993). While *Asplenium laetum* is a rather common rupestral species growing along streams in evergreen mountain rain forests (Fig. 1), *A. delitescens* has not been collected in the Cuban territory since 1914. Even though it was described from Cuba (Maxon, 1908), no specimens were deposited in Cuban herbaria. We could only study the pictures of type specimens (US, NY) and two specimens at Stockholm (S), encompassing the total amount of known Cuban collections.

In 2004, we rediscovered a population of *Asplenium delitescens* in Eastern Cuba, giving us the opportunity to examine the chromosome number and the viability of the spores. We here present the results of our morphological, palynological, anatomical and cytological analyses of these species and their taxonomic position in regard to *Hymenasplenium* and some remarks on the



FIG. 1. Distribution of *Asplenium delitescens* (★) and *Asplenium laetum* (●) in Cuba.

gametophyte morphology of *A. delitescens*. The new combinations of Neotropical species to *Hymenasplenium* are also included.

MATERIALS AND METHODS

The first specimen of *Asplenium delitescens* was collected in San Luis region in Eastern Cuba, by Pollard and Palmer in 1902, and formed the basis of the description of this species (Maxon 1908). It was also collected twice by Ekman, in Canapú, Sierra of Nipe, in woods over limestone in 1914. In November 2004, we found six individuals in the stream of La Caridad, behind the American's house in Sierra of Nipe, protected area "Pinares de Mayarí", growing terrestrially over a concave stone with abundant soil and humus in an evergreen forest near an intermittent stream at 341 m above sea level. One of those individuals (Regalado et al. 42361 BSC, HAC, HAJB) was fragmented in three specimens deposited in HAC, HAJB, and BSC.

SPECIMENS EXAMINED.—*Asplenium delitescens*: CUBA. **Holguín:** Oriente, Sierra de Nipe, ad cañon flumin. Canapú in rupibus calcar., 18.VIII.1914, *Ekman 2533* (S); Oriente, prope Bayate, Cayo del Rey, in cañon ad flum. Canapú, 6.IX.1914, *Ekman 2760* (S); Pinares de Mayarí, Sierra de Nipe, stream near La Caridad, near to Casa del Americano, 18.XI.2004, *Regalado et al. 42361* (BSC, HAC, HAJB). HONDURAS. Toledo District, on hill top in high ridge, Branch Hills, 8.I.1945, *Gentle 5139* (MO). MEXICO. **Chiapas:** Marqués de Comillas, 6 km southeast from Ejido to Benemerito de las Américas, 9.X.1984, *Martínez 8136* (NY). **Oaxaca:** Dtto. Tuxtepec and 4 km west of Rt 175. ca. 100 m, 30.VII.1971. *Mickel 5784* (NY). *Asplenium laetum*: CUBA. **Pinar del Río:** Mil Cumbres (Chavarría Ranch), path from Mil Cumbres to La Altura, 29.III.2004, *Bécquer 81192, 81195* (HAJB). **La Habana:** Tapaste, San Rafael Hills, Callejón del matadero, 11.VI.1914, *León & Ekman 4294*, (HAC). **Cienfuegos:** San Blas, Santa Clara Prov., 16.III.1929, *J. G. Jack 7093* (AJBC). **Sancti Spiritus:** Escambray, head of the J. González stream, Cudina over limestone, *Caluff & Shelton s.n.* (BSC); Topes de Collantes, head of Caburní River, IV.1985, *Caluff 1370* (BSC); Escambray, Cudina, head of Canas River, south exposition, I.1986, *Caluff 2000, 2001* (BSC); Banao Hills, Tetas de Juana, summit, moist forest over the cliff, northern slope, 24.IX.1999, *Sánchez et al. 3025* (HAJB); Banao Hills, stream near Teta de Juana, IV.1994, *Caluff & Shelton 3642* (BSC); Banao Hills, Plátanos glen, in a dry dale, 5.1995, *Caluff & Shelton 4156* (BSC); Banao Hills, Cayajaná stream, V.1995, *Caluff & Shelton 4212* (BSC); Banao Hills, stream near the old way from Caja de Agua to Teta de Juana, 10.V.2000, *Regalado et al. 42420* (HAC); Banao Hills, Plátanos glen, in a dry dale, 10.V.2000, *Regalado 90164, 90165* (MACB). **Guantánamo:** prope villam Monte Verde dictam, Cuba Orientali, I.1859, *Wright 1026* (HAC); Santo Domingo, Sabaneta, Hibráhím Sánchez Hills, in a stream, IV.1988, *Caluff & Motito 2704* (BSC); Viento Frío, stream between Vía Mulata and Barbudo River, IV.1988, *Caluff & Motito 2734* (BSC); Sierra del Purial, Viento Frío, banks of Barbudo River, IV.1992, *Caluff & Shelton 3136* (BSC); Viento Frío, stream behind the farmhouse, IV.1992, *Caluff*

& Shelton 3168, 3223 (BSC); Cuba Mountain slope, directly south of Jagüey, Yateras, Oriente; 420–510 m alt. on ground in rocky forest, rare, 24.IV.1907, Maxon 4158 (S); Bayate, XII.1928, *Hioram Hno.* 6990 (HAC); Bayate, XII.1923, *Hioram Hno.* 9322 (HAC); Bayate, Mountains of Guantánamo (Oriente), XII.1923, *Hioram Hno.* 16180 (HAC); Banks of Jaguaní River, between Cocalito and Los Lirios rivers, 100–160 m., 15.II.2004, Caluff 42385 (HAC); Vázquez, stream between the coffee depulpery and Piloto River (National Park Alejandro de Humboldt), 1.IV.1999, Sánchez & del Risco 77905, 77909, 77913, 77914, 77915 (HAJB); Yunque de Baracoa, ascension and summit (National Park Alejandro de Humboldt), 22.I.2002, Sánchez et al. 79402, 79445 (HAJB); Yunque de Baracoa, slopes (National Park Alejandro de Humboldt), 23.I.2002, Sánchez et al. 79495 (HAJB); Yunque de Baracoa, over limestone, 12.1985, Caluff 1816 (BSC). **Holguín:** La Melba, III.1972, Cárdenas s.n. (HAC); Oriente, Sierra de Nipe, prope flumin Canapú, ad rupibus calcar., 1.VIII.1914, Ekman 2531 (S); Oriente, Bayate, Cayo del Rey, in the cañon flumin Canapú, ad rup. calcar., 6.IX.1914, Ekman 2749a (S); La Melba, Palmares Stream over ultramaphic rocks, I.2001, Sánchez et al. 78652, 78695 (HAJB); National Park Alejandro de Humboldt, Arroyo Bueno, La Melba, Facistor Stream (tributary of Jaguaní River), 5.II.2001, Sánchez et al. 78844 (HAJB). **Santiago de Cuba:** Loma del Gato, Sierra Maestra, 1923, Clemente Hno. 757 (S); Loma del Gato, 850 m, VIII.1923, Clemente Hno. 784 (HAC); Ravin d'Arménie, roches Loma del Gato, 800 m, 6.1924, Clemente Hno. 1194 (HAC); III Frente, El Julio, La Pimienta, Pozo Prieto, II.1991, Caluff & Shelton 3022 (BSC); S. Maestra, south of Turquino Edge Cardero Hill 2600 feet alt., 31.VII.1935, Roig et al. 6612 (HAC); Sierra del Cobre, Loma San Juan, VII.1928, *Hioram Hno.* 7127 (HAC); Loma San Juan, XII.1923, *Hioram Hno.* 9321 (HAC); Sierra Maestra, edge of Sierra Maestra between Alcarraza and Punta de Lanza, IV.1971, Bisse & Lippold 19634 (HAJB). **Prov. unknown:** Eastern Cuba (Lesquereux Collection), I.1859, Wright 1086 (HAC).

Digital specimen images at the Herbarium Berolinense (B) (Röpert 2000) were consulted.

SAMPLES FOR STUDYING EPIDERMIS, INDUSIA AND STIPE CROSS SECTIONS.—***Asplenium delitescens*:** Regalado et al. 42361 (HAC), Ekman 2533, 2760 (S), Mickel 5784, Martínez 8136 (NY), Gentle 5139 (MO); ***A. laetum*:** Caluff 42385 (HAC), Sánchez & del Risco 77905, 77909, 77915, Sánchez et al. 79402, Bécquer 81192, 81195 (HAJB).

SAMPLES FOR STUDYING SPORES (In bold specimens studied at Scanning Electron Microscope (SEM)).—***Asplenium delitescens*:** Regalado et al. 42361 (HAC), Ekman 2760 (S); ***A. laetum*:** Wright 1026, 1086, Regalado et al. 42420 (HAC), Sánchez et al. 79402, 79495, Sánchez & del Risco 77905, 77909, Bécquer 81192, 81195 (HAJB), Regalado 90164, 90165 (MACB), Maxon 4158 (S).

Cytology.—For the cytological analysis, immature sporangia of *A. delitescens* were fixed in the field with glacial acetic acid/ethanol (1:3) and conserved at 4 °C until the processing for observing chromosomes in meiosis. The samples were gathered in Pinares de Mayarí, Sierra de Nipe, stream of La

Caridad, near American's house, 18 November 2004, 14:00 hours and 17 October 2005, 15:35 hours.

Sporangia were washed in 95% ethanol for 20 minutes, stained with Wittman's Hematoxylin for 12–24 hours, washed in glacial acetic acid for 5 minutes and mounted in Hoyer's mounting medium (Soriguer *et al.*, 1993).

Micromorphology.—Samples for studies of epidermis, indusia, and spores are listed after the examined specimens. Surfaces of spores were studied in dry samples from herbarium specimens fixed on stubs with double-sided tape and coated with gold palladium (Au/Pd, c. 20 nm) and examined using a SEM Jeol JSM 25 S-11 in the Laboratory of Palynology of the Swedish Museum of Natural History and a SEM Hitachi S-3000-N at the Real Jardín Botánico de Madrid. Values of stomata and spore length (major equatorial diameter) are reported as the average of 30 measures per sample; sizes are expressed as minimum, mean and maximum length. Terminology proposed by Punt *et al.* (1994) was followed for descriptions.

Anatomy of stipe cross sections.—Stipes were distally cut, about 2 mm below the basal pair of pinnae. Materials used for sectioning are cited after the examined specimens. They were fixed in formalin-acetic acid-alcohol solution and free-hand cross sections were made with razor blades. Petioles were cleared in 3% NaOCl solution for 3 minutes and were washed in distilled water for 2–3 minutes. Sections were stained with toluidine blue and mounted in permanent slides. For section descriptions, the terminology of Lin and De Vol (1977) was followed.

Spore sowing and gametophyte development.—Spores for cultures were taken from a sporophyte of *A. delitescens* (Regalado *et al.* 42361HAC). Spores were sown on mineral agar (Dyer, 1979) in Petri dishes (6 cm diameter) for the study of germination percentage and first stages of prothallial development. The cultures were kept in a growth chamber at 20°C at 12 hours of illumination with fluorescent tubes ($28 \mu\text{Em}^{-2}\text{s}^{-1}$)/12 hours dark overnight. These cultures were maintained with enough humidity to ensure sexual contact among the gametophytes. Gametophytes were stained with chloral hydrate acetocarmine (Edwards and Miller, 1972) and mounted in water for the morphological study. The classification of Nayar and Kaur (1968) for spore germination and the categorization of gametophyte type of development from Nayar and Kaur (1971) were followed.

RESULTS

External morphology.—*Asplenium delitescens* and *A. laetum* share several morphological characters as having creeping rhizomes, swollen petiolar bases, 1-pinnate herbaceous laminae with scattered filiform scales over the rachises; veins 1–2 forked, sori occasionally diplazioid (Table 1). They differ in following characters: *Asplenium delitescens* has dull greenish brown petioles, lanceolate pinnae, and deltate, abruptly reduced laminae ending in a deltate apical portion, whereas *A. laetum* has lustrous dark brown petioles, trapezoid

TABLE 1. Morphological characters of *Asplenium delitescens* and *A. laetum*.

Morphological characters		<i>A. delitescens</i>	<i>A. laetum</i>
Rhizome	shape	creeping	short creeping
	diameter	~4 mm	2.5–3.5 mm
Leaves	color	reddish brown	reddish brown
	shape	linear-lanceolate	linear-lanceolate
	scales size (length × width)	0.5–2.5 × 0.4–2 mm	1.7–3.5 × 0.1–0.5 mm
	position	distant from each other	near to each other
	length	22–65 cm	13.6–55.3 cm
Petiole	color	dull greenish brown or stramineous	lustrous dark brown
	length	9–32 cm	3–18.8 cm
Blade	diameter	1.5–2 mm	0.6–1.5 mm
	degree of division	pinnate	pinnate
	shape	ovate to deltoid	lanceolate to oblong-lanceolate
	apex	attenuate, pinnatifid	acuminate, entire, often broken
	base	truncate, sometimes obtuse	mostly obtuse to truncate
	size (length × width)	13–33 × 12–28 cm	10.3–36.5 × 3.5–8.5 cm
	texture	herbaceous	herbaceous
Rachis	color	greenish	brown
	indumentum	brownish to blackish filiform scales and scarce pluricellular hairs	brownish to blackish filiform scales
Pinnae	shape	lanceolate	trapezoid dimidiate
	orientation with respect to rachis	ascending	patent
	apex	acute to attenuate	acute or obtuse
	petiolule	petiolulate	shortly petiolulate
	margin	denticulate	biserrate or crenate-serrate
	size (length × width)	7–12 × 1–2 cm	2–4.6 × 0.6–1.8 cm
	number of pairs	6–11	10–23
	veins	2-forked	1–2-forked
	shape	linear	linear
	number of pairs	5–8	1–8
Venation	number of pairs	4–10 mm	2–7 mm
	length	medial	supramedial
Sori	position	light brown	light brown
	color	entire	entire
	margin	entire	entire
Indusium	margin	entire	entire
	width	0.3–0.6 mm	0.3–0.5 mm

dimidiate pinnae, and lanceolate or oblong-lanceolate, gradually reduced laminae with fragile pinnatifid apical portions (Fig. 2).

Micromorphology.—Both species have similar patterns in structure of scales, epidermis, indusia and spores. Typical features of Aspleniaceae are clathrate petiole scales with glandular tips when young. These are reddish brown, filiform linear-lanceolate in both species, but have entire margins in *A. laetum* and some marginal projections ending in glands in *A. delitescens* (Fig. 3). Epidermal cells have undulate anticlinal walls in each surface, and stomata are mainly basipolycytic. Anticlinal walls of the subsidiary cells are straight in *A. laetum* while they are slightly sinuous in *A. delitescens*. Stomata length is

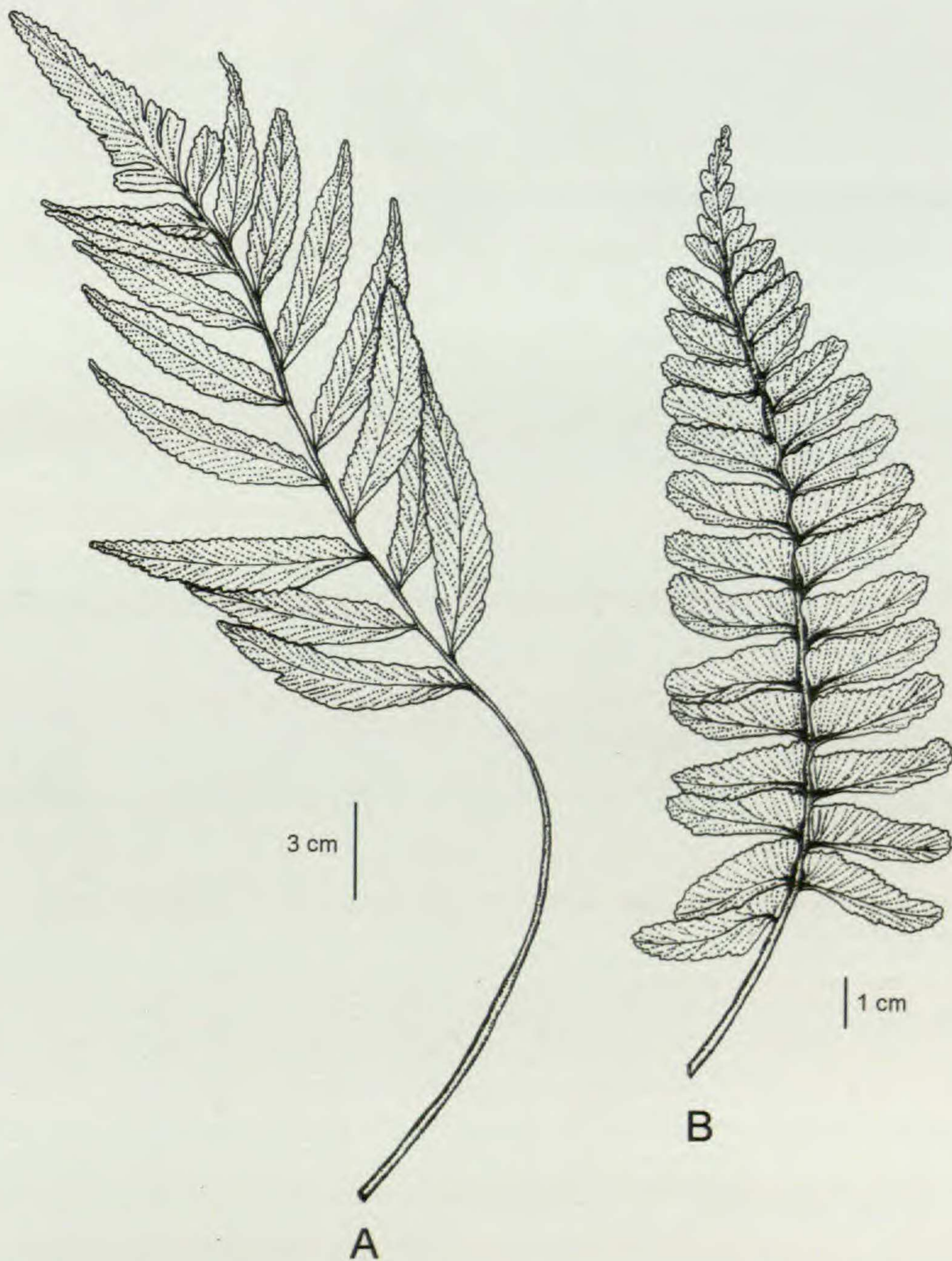


FIG. 2. Silhouettes of leaves of *Asplenium delitescens* (A) and *A. laetum* (B).

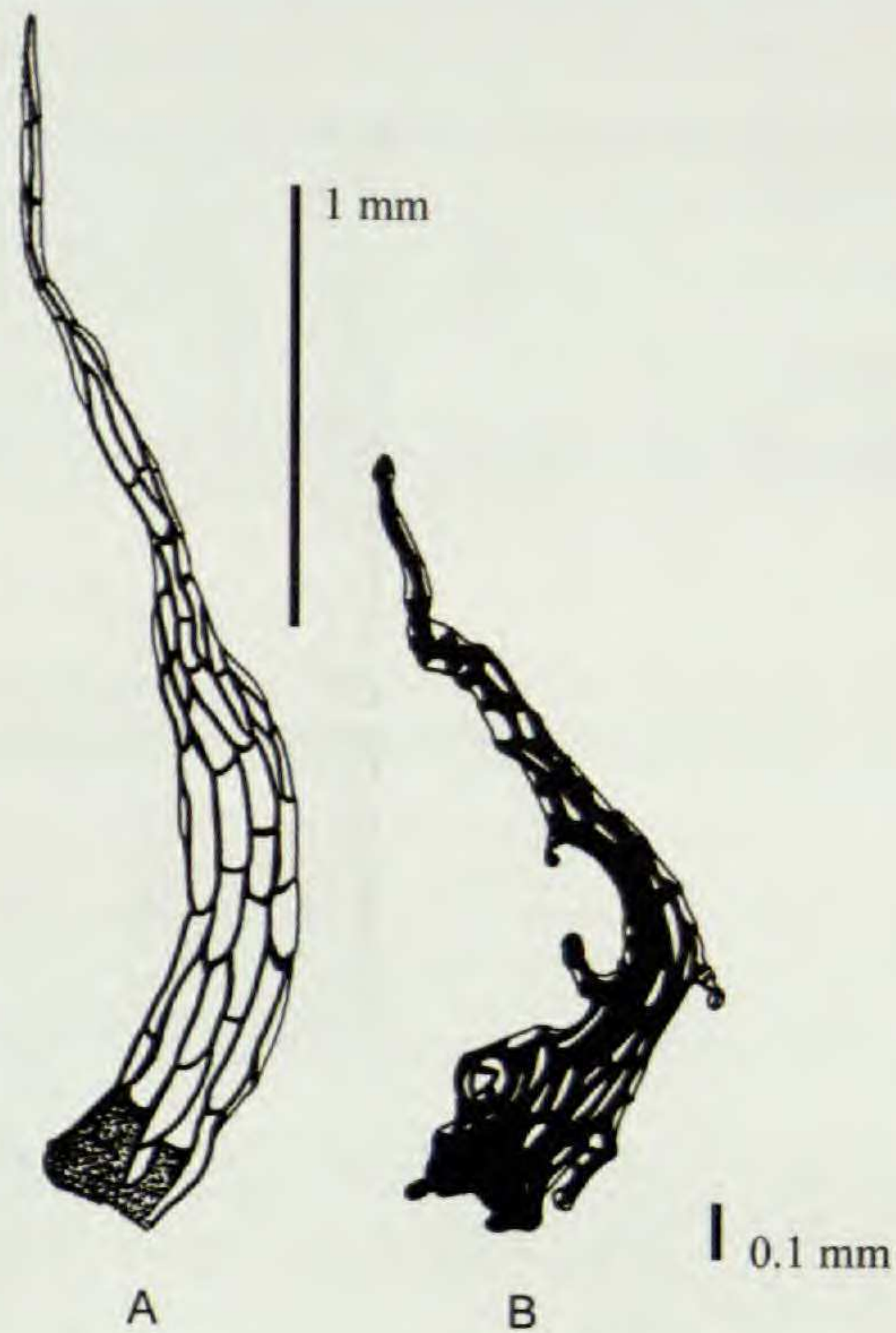


FIG. 3. Stem scales, cell structure. A. *Asplenium laetum* (78844, HAJB). B. *A. delitescens* (41361, HAC).

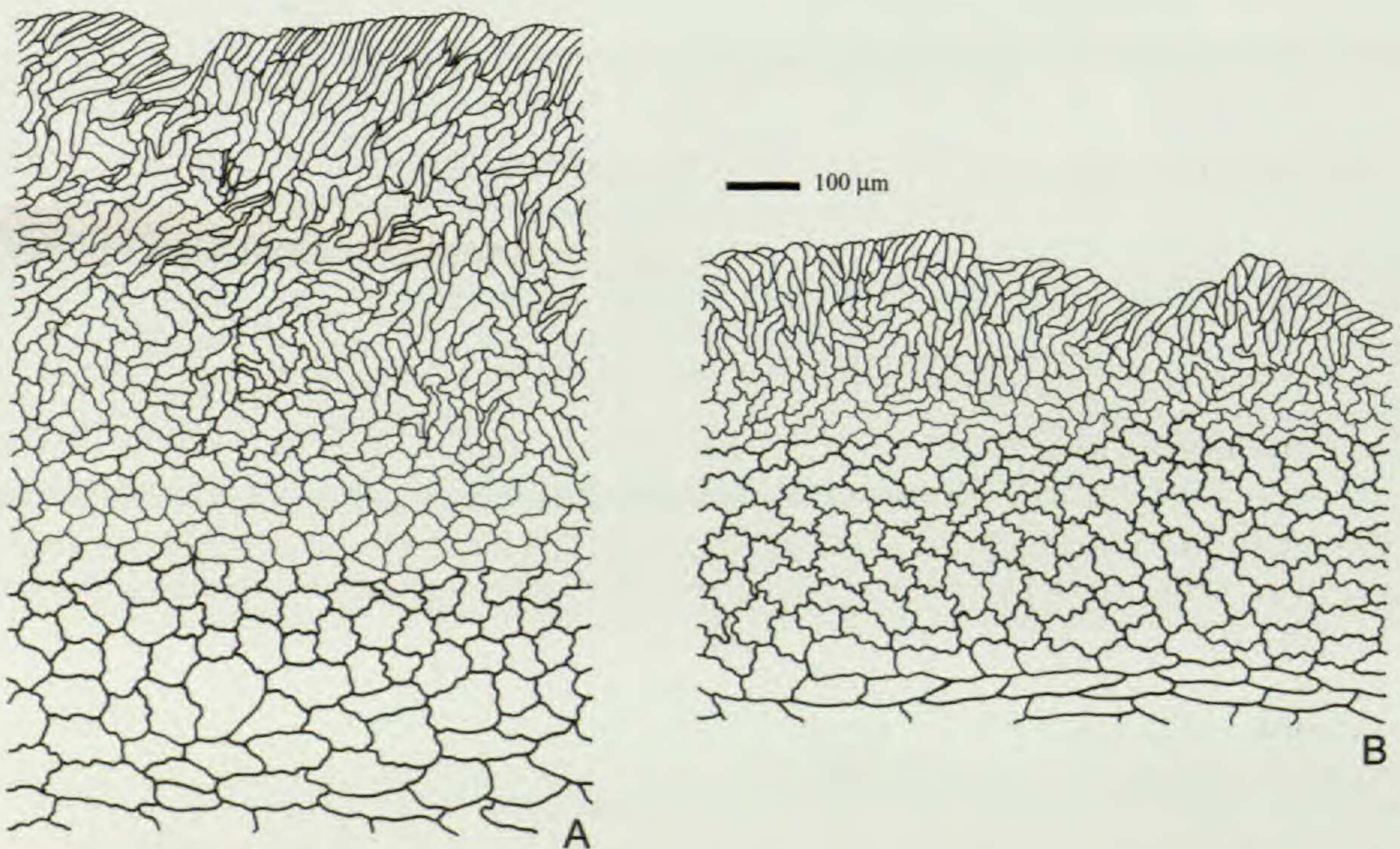


FIG. 4. Indusium. A. *Asplenium laetum* (79402, HAJB). B. *A. delitescens* (42361, HAC).

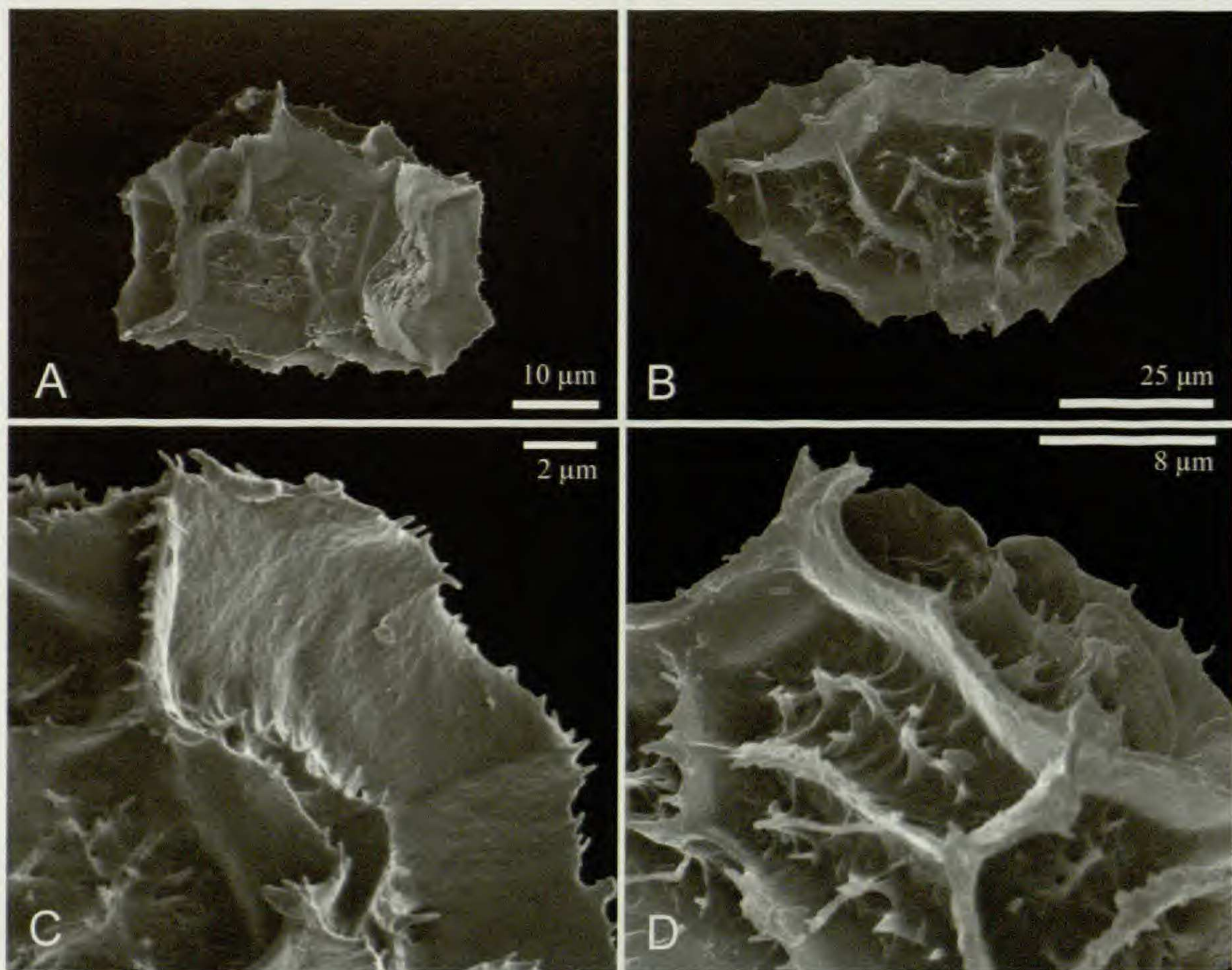


FIG. 5. Scanning electron micrographs of spores. A, C. *Asplenium delitescens* (2760, S). B, D. *A. laetum* (77909, HAJB).

(70–) 96.4 (–130) μm in *A. laetum* and (75–) 85.8 (–90) μm in *A. delitescens*. Abaxial surface is smooth in both species.

Indusia are structured into two different zones. The distal zone is composed of rectangular cells with slender anticlinal walls, irregularly situated. Proximal cells are rather radial symmetric and they are arranged with their major axis parallel to the indusial margin (Fig. 4). Upper indusium surface is also smooth in these species.

The spores of both species have an echinolophate perispore, with slim folds forming regular lacunae in *A. delitescens* and irregular ones in *A. laetum*. The surface of the lacunae is rugulate to microechinate (Fig. 5). Mean values of spore length measured along the major equatorial diameter were (30–) 34 (–35) μm in *A. delitescens* and (30–) 35.7 (–45) in *A. laetum*. Most of the examined samples of *A. laetum* presented abortive sporangia and spores.

Stipe anatomical characters.—Stipes of both species have oval transverse sections with a deep adaxial groove (Fig. 6). From periphery to center, stipe sections have a monostratified epidermal layer, with uniform polygonal isodiametric cells. The epidermis is covered with a thin and smooth cuticle. The ground tissue consists of several regularly thin-walled cells of parenchyma. A sclerenchyma zone of 4–5 cell layers is found in the abaxial cortex, near

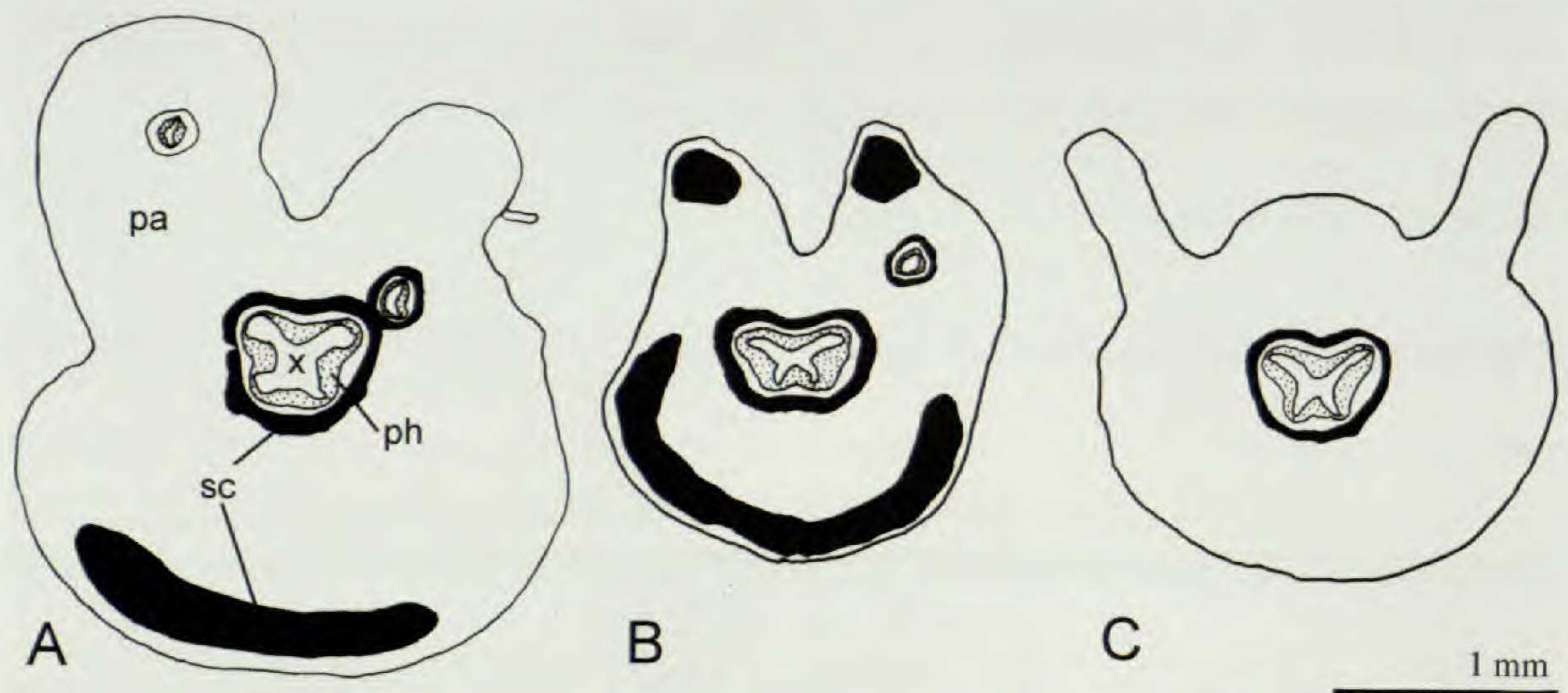


FIG. 6. Stipe cross sections of A. *Asplenium delitescens* (42361, HAC). B. *Asplenium laetum* (81195, HAJB). C. *Asplenium abscissum* (Caluff 42387, HAC). pa: parenchyma. sc: sclerenchyma. ph: phloem. x: xylem.

to the epidermis. Sclerenchyma also forms a ring that surrounds the central vascular bundle. One to two small bundles can be found in the adaxial groove sides. These bundles are enclosed by a single-layered endodermis. An internal X shaped xylem strand (curved-shaped xylem strand in smallest bundles) is surrounded by phloem (Fig. 6) and parenchyma.

Cytology.—Meiosis in *Asplenium delitescens* was studied in four spore mother cells, from a plant fixed on 17 October 2005 and showed 78 regular pairs (Fig. 7), being tetraploid, with basic chromosome number of 39.

Spore germination and gametophyte development of Asplenium delitescens.—Spore germination rate was 20%, which can be considered a low percentage. One reason for this fact could be that spores were somewhat

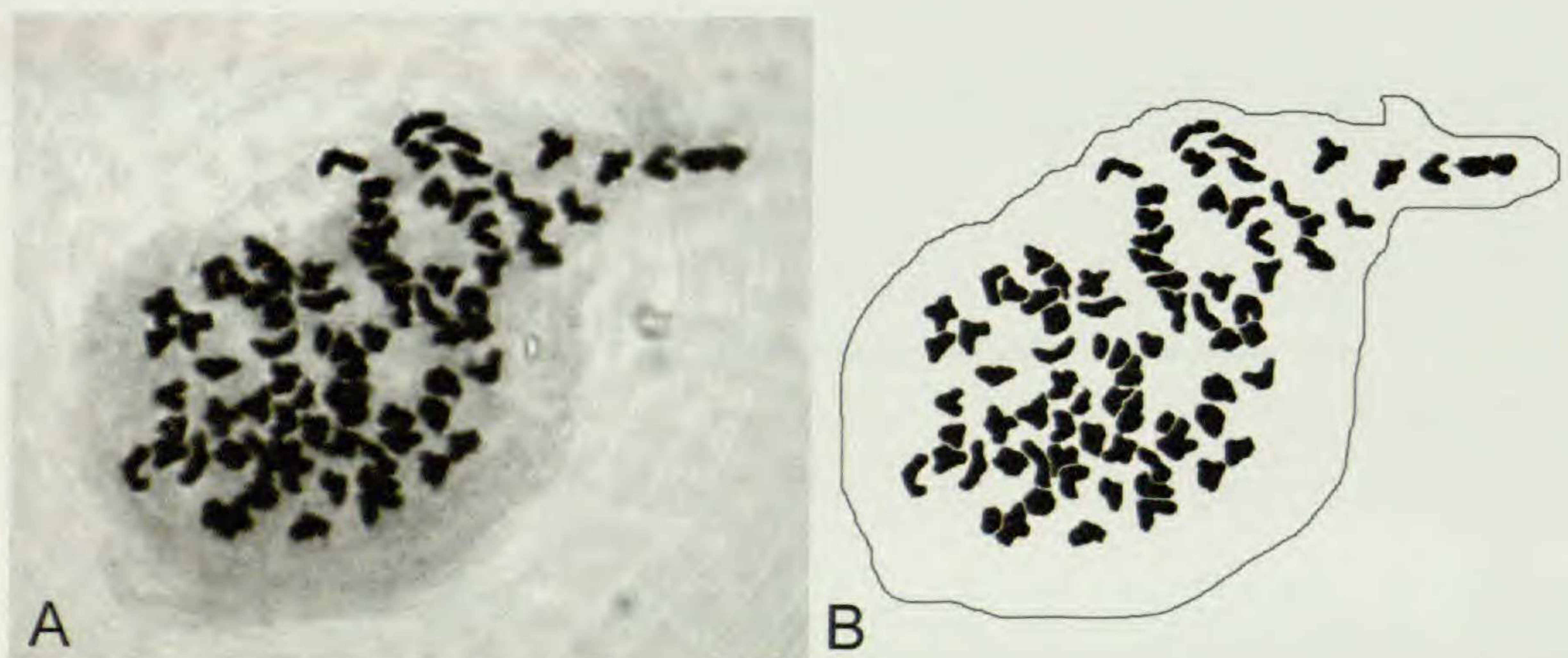


FIG. 7. Chromosomes in meiosis of *A. delitescens* (42361, HAC). A. Picture of spore mother cell (1000 X). B. Explanatory diagram. Some of the bivalents are shown partially because of superimposition.

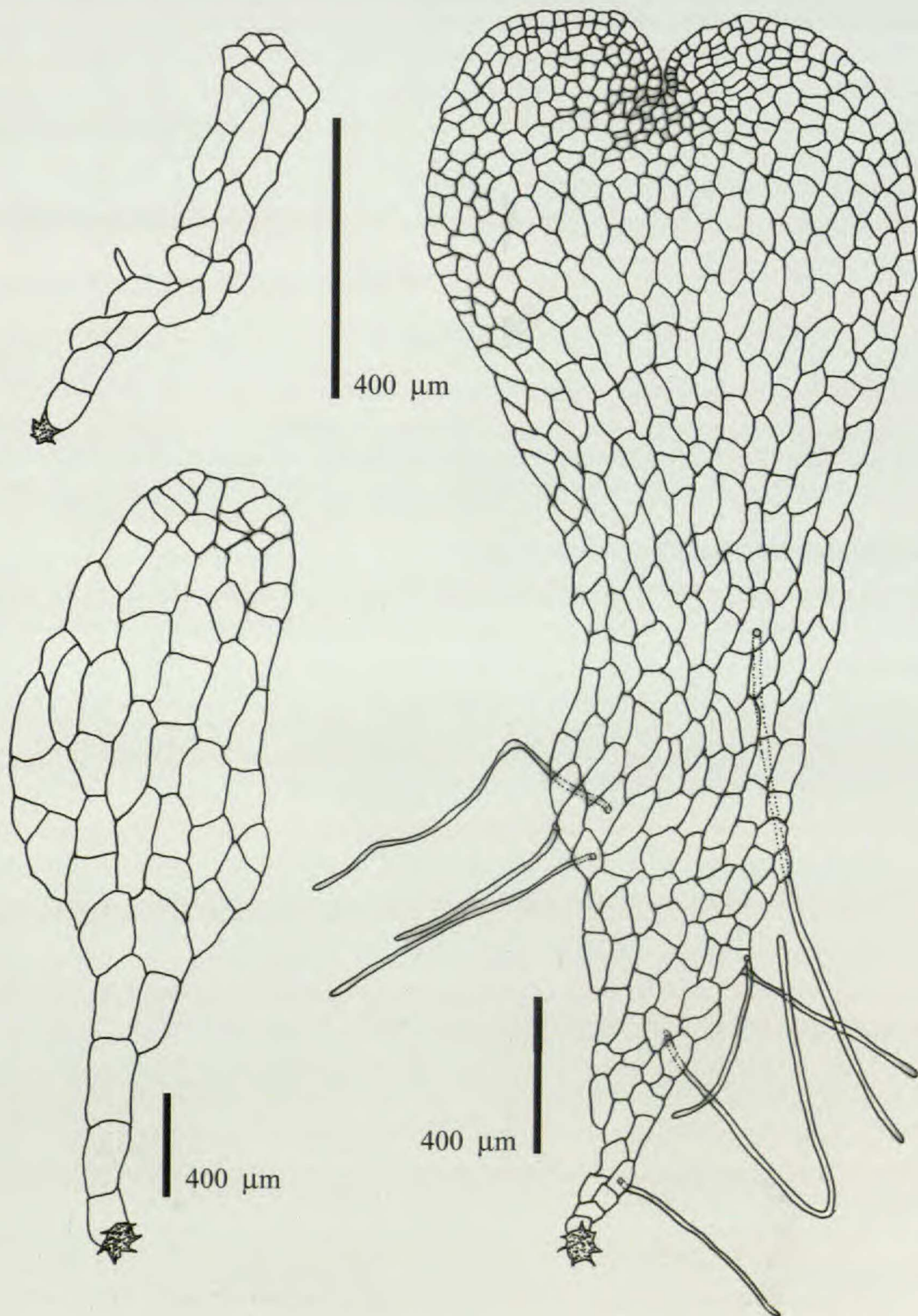


FIG. 8. First stages of gametophyte morphology of *A. delitescens* (42361, HAC).

immature at time of harvest. Spore germination follows the *Vittaria* type and the gametophyte pursues the *Adiantum* type of development, according to the classification of Nayar and Kaur (1968, 1971). A cuneiform submarginal cell originates the meristem; the bidimensional phase starts with the longitudinal

division of a subapical cell in a filament of two or three cells. Mature prothalli have the typical cordate shape, a few longer than broad in first stages, and gametangia of both sexes occur mixed in the central stripe (Fig. 8). Gametophyte morphology of *H. delitescens*, observed from lab cultures reveals the typical cordate-shaped gametophytes, characteristic of terrestrial species, according to Farrar *et al.* (2008). Although both antheridia and archegonia of normal aspect were formed in gametophytes of *A. delitescens*, no sporophytes were produced in cultures after two years from spore sowing.

DISCUSSION

External morphology and stipe anatomy.—Characters shared by *Asplenium delitescens* and *A. laetum* are creeping (always short creeping in *A. laetum*) rhizomes, swollen petiole bases, and occasionally diplazioid sori, which are also defining characteristics of the genus *Hymenasplenium* (Iwatsuki, 1975). Other characters that relate these species to *Hymenasplenium* are the adaxially shallowly grooved rachises and costae, which were first observed in Asiatic species by Iwatsuki (1975). Murakami and Moran (1993) used this character in a comparison between the Neotropical species *A. delitescens* and *A. abscissum* Willd. These species have very similar laminar architecture and pinnae shape and can be confused if the rhizomes are absent. *Asplenium abscissum*, as other *Asplenium* species, has erect rhizomes and rounded cross sections of stipes, rachises and costae even in dried specimens, with adaxial wings of parenchyma, while *A. delitescens* and *A. laetum* have adaxially grooved, not winged, stipes, rachises and costae (Fig. 6).

Spores and chromosome number.—A common spore ornamentation pattern (Murakami and Moran, 1993) was observed in both species. Most of the Cuban specimens of *Asplenium laetum* are sterile hybrids producing a high percentage of aborted spores. From 12 examined samples, only three specimens Maxon 4158 (S) and Sánchez *et al.* 79402, 79495 (HAJB) show some well-formed spores. Murakami and Moran (1993) cited 16 specimens from Belize, Costa Rica, Venezuela, Colombia, Ecuador, Peru and Bolivia as presumable hybrids, having intermediate morphology between *Asplenium delitescens* and *A. laetum*, most of them with aborted spores. However, 10 Cuban specimens (Wright 1026 (G, K, L, MO, NY, S, UC) and Wright 1086 (BM, G, K)) were cited by Murakami and Moran (1993) as *Asplenium laetum*, not under the hybrid section of their monograph. Our examined samples of Wright 1026, 1086 (HAC) had collapsed sporangia and malformed spores. Since all these specimens probably belong to the same populations, this fact suggests that normal and hybrid specimens can be found living together.

From 24 described species of the genus/sect. *Hymenasplenium*, 67% have been checked for their chromosome number: 50% of them have $x=39$, 13% show $x=36$ (*A. laetum*, *A. triquetrum* N. Murak. & R. Moran and *H. costarisorum* N. Murak. & X. Cheng), 4% possess $x=38$ (*H. subnormale* Copel.) and 33% remain unchecked. This unchecked percentage belongs mainly to Neotropical species. Our chromosome counts of $x=39$ for *Asplenium*

delitescens agree with the reported number for *Asplenium repandulum* Kunze (Smith and Mickel, 1977, rectified by Murakami, 1995 as *A. riparium* Liebm.), *Hymenasplenium cataractarum* (Rosenst.) N. Murak., *H. hondoense* (N. Murak. & Hatanaka) T. Nakaike and *H. obliquissimum* Hayata (Mitui *et al.*, 1989), *H. cardiophyllum* (Hance) T. Nakaike (Kato *et al.* 1990), *A. excisum* C. Presl, *H. apogamum* (N. Murak. & Hatanaka) T. Nakaike, *H. cheilosorum* (Kunze ex Mett.) Tagawa, *H. latipinnum* N. Murak. & X. Cheng, *H. laterepens* N. Murak. & X. Cheng and *H. obscurum* (Blume) Tagawa (Cheng and Murakami, 1998). The reports of a sexual diploid cytotype of *Asplenium laetum* from Jamaica (John Crow Mountains) in two specimens with $2n=72$ and $n=36$ (Walker, 1966) and a tetraploid $2n=144$, $n=36$ for *A. triquetrum* in Misiones, Argentina (Guillén and Daviña, 2005), are divergent from the characteristic $n=39$ usually found in the genus/sect. *Hymenasplenium* and would speak in favor for keeping *Asplenium*. Cheng & Murakami (1998), also reported sexual diploids ($n=36$) and tetraploids ($n=72$) of *H. costarisorum* from southwestern China. However, regarding the position of *H. costarisorum* and *A. laetum* in the molecular phylogeny obtained from *rbcL* sequences by Murakami (1995), the chromosome number $x=36$ can not be considered a plesiomorphic state of this character in *Hymenasplenium*, but could be a reversion or a convergence from $x=39$ to $x=36$, as it was interpreted by Cheng and Murakami (1998).

Murakami and Moran (1993) stated that *Asplenium laetum* is the most common and widely distributed species in the New World, ranging from Mexico to northern Argentina. They identified some variation correlated with geography in continental Neotropical territories, in characters such as pinnae apex and pinnae margins, and affirmed that future studies could reveal that *A. laetum* consists of several species. In Cuba, *Asplenium laetum* could hybridize with other species of the genus *Asplenium*, which seems possible because they share the same chromosome base number, considering the report of $n=36$ for Jamaican specimens (Walker, 1966) or with *Asplenium delitescens*, although different chromosome base numbers could result in a lower probability of hybridism. However, there is no morphological evidence of intermediate characters in Cuban examined specimens of *A. laetum* that permit identification of probable parental species. Putative hybrids of *A. laetum* occur equally frequently in the three distinct ranges in Cuba (Fig. 1). Distances among the ranges and with other islands of the Greater Antilles or the continental areas of Central America are not a barrier to the high dispersal capacity of fern spores (Tryon, 1970, 1979); therefore any continental or Greater Antilles species has the same opportunity of being one of the parental species of these hybrids. In addition, phylogenies of *rbcL* sequences by Murakami and Schaal (1994) and Murakami *et al.* (1999) only reflect the maternal line, and samples may not been checked for putative hybridism. A cytological and nuclear phylogenetic study, covering the whole distribution area of *A. laetum*, is needed to validate an expected different position of this species, based on a hypothesis of hybrid origin. These analyses including more species would allow also us to

understand whether the $n=36$ reported for some *Hymenasplenium* species is a reversion or a convergence from $x=39$ to $x=36$.

Taxonomical Section.—In this work we agree with previous studies (Murakami, 1995; Murakami *et al.*, 1999; Schneider *et al.*, 2004 and Smith *et al.*, 2006) to recognize *Hymenasplenium* at a generic rank. The formal combination to *Hymenasplenium* of all 11 Neotropical species is presented below.

Hymenasplenium laetum (Sw.) L. Regalado & C. Prada, **comb. nov.** \equiv *Asplenium laetum* Sw., Syn. Fil.: 79: 271. 1806. TYPE. — [icon] Schkuhr, 24: 103. Kl. Linn. Pfl.-Syst. t. 70. 1806–1809 (neotype: [designated by Proctor 1985: 371]). = *Asplenium salicifolium* var. *krugii* H. Christ, Bot. Jahrb. Syst. 24: 103. 1897. TYPE.—CUBA. **Guantánamo:** “ad Jagüey“, 500 m, “In silvestr.”, VIII.1889, *Eggers 4927* (lectotype: [designated by Sánchez and Regalado 2003: 32] P [photo]).

DISTRIBUTION.—Mexico, Continental Tropical America, Greater and Lesser Antilles, Trinidad, Tobago, Tropical Africa and Madagascar.

Hymenasplenium delitescens (Maxon) L. Regalado & C. Prada, **comb. nov.** \equiv *Diplazium delitescens* Maxon, Contr. U.S. Natl. Herb. 10: 497, t. 56, f. 1. 1908. \equiv *Asplenium delitescens* (Maxon) L. D. Gómez, Brenesia 8: 52. 1976. TYPE.—CUBA. **Santiago:** “Vicinity of San Luis”, 15–18.II.1902, *Pollard & Palmer 348* (holotype: US 403261 [photo]; isotypes: MO 1875901 [photo], NY 127304 [photo]).

DISTRIBUTION.—Mexico, Continental Tropical America and Cuba.

Hymenasplenium hoffmannii (Hieron.) L. Regalado & C. Prada, **comb. nov.** \equiv *Asplenium hoffmannii* Hieron., Hedwigia 60: 258. 1919. TYPE.—COSTA RICA. **Aguacate:** VIII.1857, *Hoffmann 836* (holotype: B [photo]; isotype: NY 149237 [photo]).

DISTRIBUTION (as reported by Murakami and Moran, 1993).—Mexico, Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica, Panamá, Colombia, Venezuela and Trinidad.

Hymenasplenium obtusifolium (L.) L. Regalado & C. Prada, **comb. nov.** \equiv *Asplenium obtusifolium* L. Sp. Pl. 1080. 1753. TYPE.—Petiver, Pter. Amer. 117, t.2, fig 14. [Plumier, Traité Foug. Amér. t.67.1705] (erroneously cited as f. 4 in the original description). Based on a plant from Morne de la Calebasse, Martinique.

DISTRIBUTION (as reported by Murakami and Moran, 1993).—Panamá, Jamaica, Puerto Rico, Lesser Antilles, Colombia, Venezuela and Trinidad.

Hymenasplenium ortegae (N. Murak. & R. C. Moran) L. Regalado & C. Prada, **comb. nov.** \equiv *Asplenium ortegae* N. Murak. & R. C. Moran, Ann. Missouri Bot. Gard. 80: 23, fig. 11 b, c. 1993. TYPE.—VENEZUELA. **Paéz:** Apure, Campamento de Corpo Andes, along Río Arauca at Colombian border, near Torunos. 200 m. (656.2 ft.), 29.VI.1983, *van der Werff & González 4599* (holotype: MO n.v.; isotype: NY 149265 [photo]).

DISTRIBUTION (as reported by Murakami and Moran, 1993).—Venezuela, Colombia, Ecuador, Peru and Brazil.

Hymenasplenium purpurascens (Mett. ex Kuhn) L. Regalado & C. Prada, **comb. nov.** \equiv *Asplenium purpurascens* Mett. ex Kuhn, Linnaea 36: 102. 1869. TYPE.—ECUADOR. **Chimborazo:** “ad pedem montis Chimborazo”, *Spruce 5697* (holotype: B [photo]).

DISTRIBUTION (as reported by Murakami and Moran, 1993).—Ecuador.

Hymenasplenium repandulum (Kunze) L. Regalado & C. Prada, **comb. nov.** \equiv *Asplenium repandulum* Kunze, Linnaea 9: 65. 1834. TYPE.—PERU. **Huánuco:** Pampayaco, in sylvis montosis ad arborum truncos, VII.1829, *Poeppig s. n.* (holotype: B n.v.; isotype: NY (a pinna) 149290 [photo]).

DISTRIBUTION (as reported by Murakami and Moran, 1993).—Ecuador and Peru.

Hymenasplenium riparium (Liebm.) L. Regalado & C. Prada, **comb. nov.** \equiv *Asplenium riparium* Liebm. Mexic. Bregn. (seors. 92): 244. 1849. \equiv *Asplenium obtusifolium* var. *riparium* (Liebm.) Domin, Pterid. Isl. Dominica, Rozpr. Král. České Spolecn. Nauk. Tr. Mat.-Prir., Nov. Rad. 2: 175. 1929. TYPE.—MEXICO. **Veracruz:** Hacienda de Jovo, *Liebmann s. n.* [Pl. Mex. 310] (lectotype [designated by Smith 1981: 51]: C n.v.).

DISTRIBUTION (as reported by Mickel and Smith, 2004).—Mexico, Guatemala, Belize, Honduras, Nicaragua, Costa Rica, Panama, Colombia, Venezuela and Ecuador.

Hymenasplenium triquetrum (N. Murak. & R. C. Moran) L. Regalado & C. Prada, **comb. nov.** \equiv *Asplenium triquetrum* N. Murak. & R. C. Moran, Ann. Missouri Bot. Gard. 80: 31, fig. 8 b, c. 1993. TYPE.—BOLIVIA. La Paz: Prov. **Nor-Yungas:** Polo-Polo bei Coroico [Im tiefen Schatten des Hochwaldes oder auf nassem Boden], 1100 m, 10.XI. 1912, *Buchtien 625* (holotype: MO n.v.; isotypes: NY [photo], BM, K, Z n.v.).

DISTRIBUTION (as reported by Murakami and Moran, 1993).—Bolivia and Brazil.

Hymenasplenium volubile (N. Murak. & R. C. Moran) L. Regalado & C. Prada, **comb. nov.** \equiv *Asplenium volubile* N. Murak. & R. C. Moran, Ann. Missouri

Bot. Gard. 80: 32, fig. 13 e. 1993. TYPE.—ECUADOR. **Cotopaxi**: Quevedo-Latacunga road, km 46 from Quevedo, NE exposed slopes with rainforest, 600 m, 0°55'S, 79°11'W, 4.IV.1973, *Holm-Nielsen et al.* 2905 (holotype: 2914385 MO [photo], isotypes: AAU, F, UC n.v.).

DISTRIBUTION (as reported by Adams, 1995).—Costa Rica, Panama, W Colombia, W Ecuador.

Hymenasplenium basiscopicum (R. C. Moran & M. A. Sundue) L. Regalado & C. Prada, **comb. nov.** \equiv *Asplenium basiscopicum* R. C. Moran & M. A. Sundue, *Brittonia* 56: 124. 2004. TYPE.—BOLIVIA. **Santa Cruz**: Prov. Ichilo, Parque Nacional Amboró, steep slopes above and 1 km S of Río Saguayo, 750 m, 17°41'S, 63°44'W, 20.I.1988, *M. Nee* 36020 (holotype: NY [photo], isotypes: LPB, MO n.v.).

DISTRIBUTION (as reported by Moran & Sundue, 2004).—Bolivia.

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